

Amendments to the Specification

Please replace the paragraph beginning at page 1, line 15, with the following rewritten paragraph:

Methods utilizing mass spectrometry for the analysis of a target polypeptide have been taught wherein the polypeptide is first solubilized in an appropriate solution or reagent system. The type of solution or reagent system, e.g., comprising an organic or inorganic solvent, will depend on the properties of the polypeptide and the type of mass spectrometry performed and are well-known in the art (see, e.g. Vorm et al. (1994) Anal. Chem. 66:3281 (for MALDI) and Valaskovic et al. (1995) Anal. Chem. 67:3802 (for ESI)). Mass spectrometry of peptides is further disclosed, e.g. in WO 93/24834 by Chait et al.

Please replace the paragraph beginning at page 37, line 3, with the following rewritten paragraph:

Figure 1 is a photograph of a tricine gel HiQ 1(Elution) comparing Insulin Resistance versus Normal;

Figure 2 is a trypsin digested spectra graph depicting the characteristic profile of the ion 1208 (SEQ ID NO:1);

Figure 3 is a photograph of a tricine gel HIQ2 (scrub) comparing Normal versus Insulin Resistance and Type I Diabetes; and

Figure 4 is a trypsin digested spectra graph depicting the characteristic profile of the ion 1447 (SEQ ID NO:3).

Please replace the paragraph beginning at page 41, line 8, with the following rewritten paragraph:

DEAE Column Protocol:

- 1) Cast 200 µl of 50% slurry;
- 2) Equilibrate column in 5 bed volumes of 50 mM tricine pH 8.8 (binding buffer);
- 3) Dissolve 25 µl of sera in 475 µl of binding buffer;
- 4) Wash column in 5 bed volumes of binding buffer;
- 5) Elute column in 120 µl of 0.4 M Phosphate buffer (PB) pH 6.1;
- 6) Elute column in 120 µl of 50 mM citrate buffer pH 4.2;
- 7) Scrub column with 120 µl sequentially with each of 0.1% ~~triton~~ TRITON, 1.0% ~~triton~~ TRITON and 2% SDS in 62.5 mM [[Tris]] TRIS pH 6.8.

Please replace the paragraph, as amended on September 22, 2003, beginning at page 41, line 22, with the following rewritten paragraph:

Butyl SEPHAROSE Column Protocol:

- 1) Cast 150 µl bed volume column;

- 2) Equilibrate column in 5 bed volumes of 1.7 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM PB pH 7.0 (binding buffer);
- 3) Dissolve 35 μl of sera in 465 μl of binding buffer and apply;
- 4) Wash column in 5 bed volumes of binding buffer;
- 5) Elute column in 120 μl of 0.4 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM PB pH 7.0;
- 6) Elute column in 120 μl of 50 mM PB pH 7.0;
- 7) Scrub column with 120 μl sequentially with each of 0.1% ~~triton~~ TRITON , 1.0% ~~triton~~ TRITON and 2% SDS in 62.5 mM [[Tris]] TRIS pH 6.8.

Please replace the paragraph, as amended on September 22, 2003, beginning at page 42, line 14, with the following rewritten paragraph:

Phenyl SEPHAROSE Column Protocol:

- 1) Cast 150 μl bed volume column;
- 2) Equilibrate column in 5 bed volumes of 1.7 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM PB pH 7.0 (binding buffer);
- 3) Dissolve 35 μl of sera in 465 μl of binding buffer and apply;
- 4) Wash column in 5 bed volumes of binding buffer;
- 5) Elute column in 120 μl of 0.2 M $(\text{NH}_4)_2\text{SO}_4$ in 50 mM

PB pH 7.0;

- 6) Elute column in 120 µl of 50 mM PB pH 7.0;
- 7) Scrub column with 120 µl sequentially with each of
0.1% ~~triton~~ TRITON , 1.0% ~~triton~~ TRITON and 2% SDS in 62.5
mM [[Tris]] TRIS pH 6.8.

Please replace the paragraph beginning at page 43, line 5,
with the following rewritten paragraph:

HiQ Anion Exchange Mini Column Protocol:

- 1) Dilute sera in sample/running buffer;
- 2) Add HiQ resin to column and remove any air bubbles;
- 3) Add ultrafiltered (UF) water to aid in column packing;
- 4) Add sample/running buffer to equilibrate column;
- 5) Add diluted sera;
- 6) Collect all the flow-through fraction in ~~Eppendorf~~
EPPENDORF tubes until level is at resin;
- 7) Add sample/running buffer to wash column;
- 8) Add elution buffer and collect elution in ~~Eppendorf~~
EPPENDORF tubes.

Please replace the paragraph beginning at page 43, line 18, with the following rewritten paragraph:

HiS Cation Exchange Mini Column Protocol:

- 1) Dilute sera in sample/running buffer;
- 2) Add HiS resin to column and remove any air bubbles;
- 3) Add UF water to aid in column packing;
- 4) Add sample/running buffer to equilibrate column for sample loading;
- 5) Add diluted sera to column;
- 6) Collect all flow through fractions in ~~Eppendorf~~ EPPENDORF tubes until level is at resin;
- 7) Add sample/running buffer to wash column;
- 8) Add elution buffer and collect elution in ~~Eppendorf~~ EPPENDORF tubes.

Please replace the paragraph beginning at page 44, line 7, with the following rewritten paragraph:

Illustrative of the various buffering compositions useful in this technique are:

Sample/Running buffers: including but not limited to Bicine buffers of various molarities, pH's, NaCl content, ~~Bis-Tris~~ BIS-TRIS buffers of various molarities, pH's, NaCl content, Diethanolamine of various molarities, pH's, NaCl content, Diethylamine of various

molarities, pH's, NaCl content, Imidazole of various molarities, pH's, NaCl content, Tricine of various molarities, pH's, NaCl content, Triethanolamine of various molarities, pH's, NaCl content, [[Tris]] TRIS of various molarities, pH's, NaCl content.

Elution Buffer: Acetic acid of various molarities, pH's, NaCl content, Citric acid of various molarities, pH's, NaCl content, HEPES of various molarities, pH's, NaCl content, MES of various molarities, pH's, NaCl content, MOPS of various molarities, pH's, NaCl content, PIPES of various molarities, pH's, NaCl content, Lactic acid of various molarities, pH's, NaCl content, Phosphate of various molarities, pH's, NaCl content, Tricine of various molarities, pH's, NaCl content.

Please replace the paragraph, as amended on September 22, 2003, beginning at page 46, line 6, with the following rewritten paragraph:

As a result of these procedures, the disease specific markers namely peroxisomal carnitine octanoyl transferase protein having a molecular weight of about 1208.6574 daltons and a sequence of SEQ ID NO:1, betaine/GABA transport protein having a molecular weight of about 1211.5591 daltons and a sequence of SEQ ID NO:2, and adrenergic, alpha 2A receptor protein having a molecular weight of about 1446.7831 daltons ~~having~~ and a sequence of SEQ ID NO:3

related to insulin resistance were found.

Please replace the paragraph beginning at page 49, line 6, with the following rewritten paragraph:

The specific disease markers which are analyzed according to the method of the invention are released into the circulation and may be present in the blood or in any blood product, for example plasma, serum, cytolized blood, e.g. by treatment with hypotonic buffer or detergents and dilutions and preparations thereof, and other body fluids, e.g. ~~[[CSF]]~~ cerebrospinal fluid (CSF), saliva, urine, lymph, and the like. The presence of each marker is determined using antibodies specific for each of the markers and detecting specific binding of each antibody to its respective marker. Any suitable direct or indirect assay method may be used to determine the level of each of the specific markers measured according to the invention. The assays may be competitive assays, sandwich assays, and the label may be selected from the group of well-known labels such as radioimmunoassay, fluorescent or chemiluminescence immunoassay, or immunoPCR technology. Extensive discussion of the known immunoassay techniques is not required here since these are known to those of ~~skilled~~ skill in the art. See Takahashi et al. (Clin Chem 1999; 45(8): 1307) for a detailed example of an assay.